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GB 2181843 A EP 0757246 A2 EP 0245994 A2
WO 98/24548 A1 WO 89/00755 A1 DE 003132926 A
US 4878597 A

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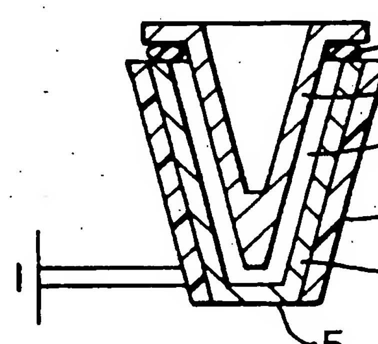
(54) Abstract Title
Reduced volume heated reaction vessel

(57) A vessel of reduced volume for biological reactions such as PCR comprises a container 1 heated by an electrically conducting polymer, eg a surrounding sheath 6 of the polymer, or by way of the lid or container body being made of the polymer. Cap 2 extends into the container to reduce the capacity thereof and define, with the container wall, a space 3 having a consistent cross section throughout of capillary dimensions eg 0.4 - 1.2 mm. The base 5 may be optically clear to allow observation of the reaction space. The cap may snap-fit and its projection may accommodate a monitoring device eg a thermocouple (13, Fig 3).

Fig.1.



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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Fig.1.

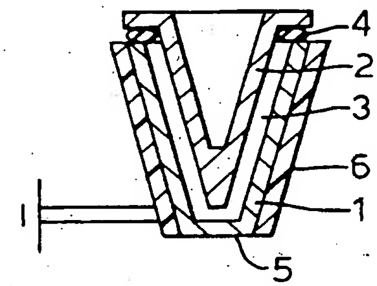


Fig.2.

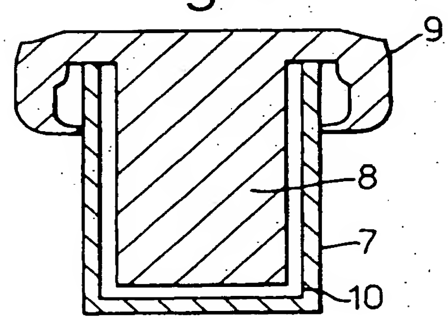


Fig.3.

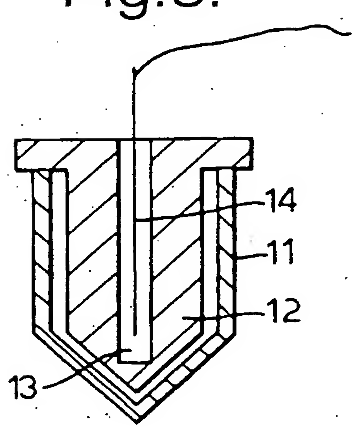


Fig.4.

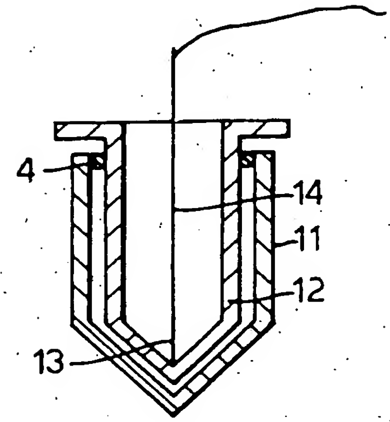
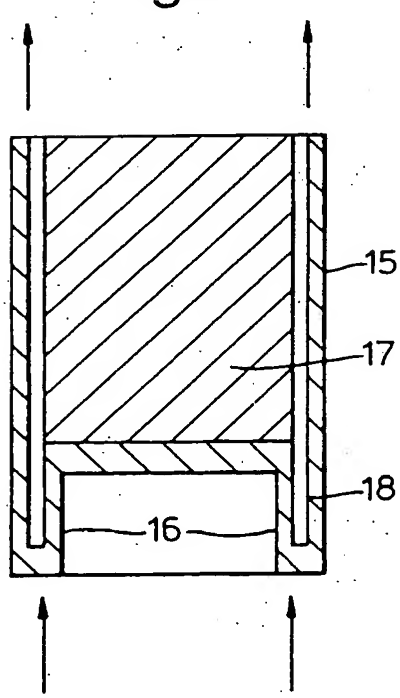


Fig.5.



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REACTION VESSELS

The present invention relates to reaction vessels and parts therefor, particularly reaction vessels adapted for use in biochemical reactions which require heating. It is also concerned with apparatus in which such reaction vessels are employed.

Typically, such reaction vessels are used in thermocycling apparatus wherein, for example, DNA amplification methods like the Polymerase Chain Reaction (PCR) can be carried out. PCR is a procedure for generating large quantities of a particular DNA sequence and is based upon DNA's characteristics of base pairing and precise copying of complementary DNA strands. Typical PCR involves a cycling process of three basic steps.

Denaturation : A mixture containing the PCR reagents (including the DNA to be copied, the individual nucleotide bases (A,T,G,C), suitable primers and polymerase enzyme) are heated to a predetermined temperature to separate the two strands of the target DNA.

Annealing : The mixture is then cooled to another predetermined temperature and the primers locate their complementary sequences on the DNA strands and bind to them.

Extension : The mixture is heated again to a further predetermined temperature. The polymerase enzyme (acting as a catalyst) joins the individual nucleotide bases to the end of the primer to form a new strand of DNA, which is complementary to the sequence of the target DNA, the two strands being bound together.

As well as amplification reactions such as PCR, biochemical reaction vessels can be used in nucleic acid sequencing reactions and in enzyme kinetic studies. In the latter, the activity of enzymes at various temperatures is studied. Other biochemical reactions, especially those involving enzymatic

activity, may have to be effected under temperatures conditions.

A preferred form of reaction vessel comprises a capillary tube. This is because capillary tube reaction vessels afford a high surface area to volume ratio which is good for thermal transfer and uniformity of heating throughout the sample. Furthermore, the volume of samples used in these reactions is frequently very small, of the order of microlitres or less and small volume vessels are thus essential.

Capillary tube reaction vessels are usually filled by allowing the sample to be drawn into the tube under capillary action. The ends of the tube are then sealed. In the case of a glass tube, which is the usual form, sealing is typically effected thermally.

This thermal sealing method has a major disadvantage in being liable to degrade the sample. Also however, a glass tube of what might well be less than 2mm outside diameter and about 4cm length, is very fragile. There are capillary reaction vessels which have one end presealed. These may be filled by employing centrifuge or vacuum techniques. These are however time consuming and besides entail a risk of retained air and contamination from air.

It is not uncommon for a newly opened box to contain four or five broken tubes in a bank of 96 such vessels, which is one popular quantity for use in biochemical thermocycling apparatus. Further breakages are very likely to occur during filling and mounting and even in use, not least because heating and cooling is typically effected using turbulent hot and cold air.

There exist also reaction vessels formed from plastics material and vessels which are not capillary in form. Such vessels typically have a maximum internal diameter of 5 to 10 mm and are conical or paraboloid tapering down to the base. These are relatively easily filled and are provided with caps

which seal thereto. They are relatively unbreakable but have the disadvantage that the required temperatures may not be accurately attained or consistently attained throughout the sample or with each cycle. Because of the low surface area to volume ratio, heat transfer is poor in conventional tubes.

Reaction vessels in which a cap for a reaction vessel projects into the vessel in order to reduce the volume thereof are described for example in EP-A-245994 and US Patent No. 4578588.

In this way, the insertion of the cap member into the vicinity of the sample results in an increase in the surface area to volume ratio of the sample, so that the required temperature can be consistently and rapidly attained throughout the reagent mass. In addition the reaction vessel which is easy to fill.

The present invention provides a reaction vessel comprising a container, a cap member, and an electrically conducting polymer which is arranged so as to heat reagents in the reaction vessel when current is supplied to said polymer, the cap member being formed so as to project into the container to reduce the capacity thereof and to create a space therebetween of substantially consistent proportions.

In this way, the insertion of the cap member into the vicinity of the sample results in an increase in the surface area to volume ratio of the sample, so that the required temperature can be consistently and rapidly attained throughout the reagent mass. In addition the reaction vessel which is easy to fill.

The expression "substantially consistent proportions" used herein means that the space, which will form the reagent volume is of substantially similar cross section throughout. This means that externally applied factors such as heating or cooling means, will be effective throughout the entire volume of the reagent in a substantially consistent manner.

The reaction vessels of the invention are heated using an electrically conducting polymer which emits heat when an electric current is passed through it, as described in co-pending International Patent Application No. PCT/GB97/03187.

Electrically conducting polymers are known in the art and may be obtained from Caliente Systems Inc. of Newark, USA. These polymers can provide temperatures up to 300° C and are therefore well suited for use in PCR processes where the typical range of temperatures is between 30° and 100°C. Other such polymers are disclosed for example in US Patent No. 5106540 and US Patent No. 5106538.

An advantage of this arrangement over a conventional block heater is derived from the fact that polymers which conduct electricity are able to heat rapidly. The heating rate depends upon the precise nature of the polymer, the dimensions of polymer used and the amount of current applied. Preferably the polymer has a high resistivity, for example in excess of 1000ohm.cm. The temperature of the polymer can be readily controlled by controlling the amount of electric current passing through the polymer, allowing it to be held at a desired temperature for the desired amount of time. Furthermore, the rate of transition between temperatures can be readily controlled after calibration, by delivering an appropriate electrical current, for example under the control of a computer programme.

Furthermore as compared to a block heater rapid cooling can also be assured because of the low thermal mass of the polymer.

In addition, the use of polymer as the heating element for the reaction vessel will generally allow the apparatus to take a more compact form than existing block heaters, which is useful when carrying out chemical reactions in field conditions such as in the open air, in a river, on a factory floor or even in a small shop.

If desired, the reaction vessel of the invention may be subjected to artificial cooling to increase the speed of cooling. Suitable cooling methods include forced air cooling, for example by use of fans, immersion in ice or water baths etc, or the use of thermoelectric devices or compressor refrigerator technologies.

The reaction vessel may take the form of a reagent container such as a glass or plastics container, with electrically conducting polymer arranged in close proximity to the container. In one embodiment of the vessel, the polymer is provided as a sheath which fits around the reaction vessel, in thermal contact with the vessel. The sheath can either be provided as a shaped cover which is designed to fit snugly around a reaction vessel or it can be provided as a strip of film which can be wrapped around the reaction vessel and secured.

The polymer sheath arrangement means that close thermal contact is achievable between the sheath and the reaction vessel. This ensures that the vessel quickly reaches the desired temperature without the usual lag time arising from the insulating effect of the air layer between the reaction vessel and the heater. Furthermore, a polymer sheath can be used to adapt apparatus using pre-existing reaction vessels. In particular, a strip of flexible polymer film can be wrapped around a reaction vessel of various different sizes and shapes.

Where a sheath is employed it may be advantageous for it to be perforated or in some way reticulated for to permit even readier access by a cooling medium if the polymer is not itself used to effect the cooling.

In a preferred arrangement, the polymer is provided as an integral part of the reaction vessel, either as part of the container or the cap member. The container and/or cap member may be made from the polymer by extrusion, injection moulding

or similar techniques. Alternatively, the container or cap member may be manufactured using a composite construction in which a layer of the conducting polymer is interposed between layers of the material, such as plastics or glass, from which the container or cap member is made. In a further alternative, the internal or external surfaces of the container and/or cap member are coated with the polymer. Alternatively, the container or cap member is basically made of the polymer coated with a thin laminate of a PCR compatible material. Such vessels may be produced using lamination and/or deposition such as chemical or electrochemical deposition techniques as is conventional in the art.

Vessels which comprise the polymer as an integral part of either the container or the cap member may provide particularly compact structures.

If several reaction vessels are required for a particular reaction, any electrical connection points can be positioned so that a single supply can be connected to all the reaction vessels or tubes. The reaction vessels may be provided in an array.

Alternatively, each of or each group of reaction vessels may have its own heating profile set by adjusting the applied current to that vessel or group of vessels. This allows for the temperature of individual vessels can be controlled independently of one another with their own thermal profile.

The polymer may suitably be provided in the form of a sheet material or film, for example of from 0.01mm to 10 mm, such as from 1 to 10 mm, and preferably 0.1 to 0.3 mm thick. By using thin films, the volume of polymer required to cover a particular reaction vessel or surface is minimised. This reduces the time taken for the polymer to heat to the required temperature as the heat produced by passing the current through the polymer does not have to be distributed throughout a large volume of polymer material.

In use, the polymer component of the reaction vessel is arranged such that an electric current can be generated within the polymer. This can either be achieved by providing the polymer with connection points for connection to an electrical supply or by inducing an electric current within the polymer, for example by exposing the polymer to suitable electrical or magnetic fields.

The close thermal contact between the polymer and the reagents or reagent container which may be established in the reaction vessels of the invention reduces or eliminates the insulating effect of the air layer between the heating element and the reaction vessel.

The control means is suitably an automatic control means such as a computer controlled interface arrangement. By using a programmable controller for the electrical circuit connected to the polymer, a defined heating regime, for example a defined number of cycles of predetermined temperature stages to be established over predetermined time intervals and dwells can be pre-programmed using the apparatus, including employing different temperature and time profiles with different reaction vessels in the same apparatus at the same time.

The control means may include a temperature monitoring device such as a thermocouple, which monitors the temperature of the reaction vessel and feeds this information into the control system so that the desired regime of heating and/or cooling is adhered to.

Alternatively, the temperature of the polymer may be monitored directly by measuring its resistivity, for example by arranging the polymer heating element as a resistor in a wheatstone bridge circuit arrangement. This avoids the use other temperature measurement devices such as thermocouples.

Optionally, the apparatus further comprises artificial cooling means such as one or more fans.

The apparatus may include a plurality of containers. The polymer may be provided as an integral part of each container, as a sheath around each container or arranged such that a layer of polymer is interposed between adjacent containers. Any electrical connection points on the polymer may be connected to a single electrical supply, if a number of reactions requiring the same temperature stages are being carried out.

However, in a preferred embodiment the apparatus is arranged such that the polymer in contact with (or forming) a container or a group of containers is connected to an individual supply, several containers or groups of containers being connected to different, independently controlled electrical supplies. With this arrangement, a number of different reactions requiring different temperature stages can be carried out at the same time as each container or group of containers has its own heating element. This arrangement allows users to carry out a number of small batch reactions using a single apparatus.

The use of a cap member to effectively reduce the volume of the container means that a larger container may be used initially. This provides for easier fill as reagent may be applied by conventional addition methods such as pouring or introduction via a pipette or other applicator, either manually or using a robotic device.

Preferably the cap member is also formed to seal the vessel. This allows the application of the cap to produce rapid sealing of the vessel. One method by which this may be achieved is to make the cap member of a plastics material which is arranged to snap fit onto the container.

The cap member may be adapted to assist in the observation or monitoring of a reaction being effected in the container. For example, a portion of the cap member which projects into the container may comprise an optical waveguide. Alternatively or additionally, a lens may be provided in the

cap member and arranged to enhance observation of contents of the container.

The container suitably comprises a glass or plastics material. Preferably, the material is transparent to allow reactions taking place therein to be observed, particularly when visible labelling methods, for example, fluorescent labels are being used.

In yet a further preferred embodiment, the distance between the cap member and the container at any point when the cap member is in place is substantially similar in proportion to the diameter of a capillary tube. In particular the distance between the surface of the cap member at any particular point in the reagent volume is suitably from 0.4 to 1.2 mm, preferably from 0.4 to 0.8mm. In this embodiment, reagent will become evenly spaced throughout the reaction volume as a result of capillary action.

The vessel may be conical or paraboloid in form or have an outwardly domed base. It may however be particularly advantageous for the vessel to be right cylindrical in form. In this case the cap member may be arranged to impinge upon the base so that the space created when the cap member is in place has the form of an open cylinder. This means that application of the desired external conditions, for example of heating or cooling, to the sides of the vessel will be conveyed conveniently and consistently to reagent in the space.

Suitably the vessel comprise a conventionally shaped reaction tube or curvette so that is may be readily fitted into conventional apparatus such as solid block heaters. In this case, the cap members may be provided separately for use in the reaction vessels.

In a preferred embodiment, the cap member is adapted to receive reaction monitoring device, such as a temperature measuring device, like a thermocouple, so that the temperature

of the reagents can be monitored throughout the reaction if required.

By means of the invention, reaction vessels are provided in which reaction temperatures can be consistently attained when traditional hot and cold gases are employed to effect the thermal cycle. However the invention affords the added advantage that the vessel may contain, or comprise, or be enveloped within a heater element, possibly with cooling being provided for at an opposite surface. Thus, for example, the cap member may comprise or contain the electrically conducting polymer heater element while the container member may be arranged for exposure to a cooling medium.

The reaction vessels of the invention allow precise temperatures to be reached and maintained for suitable time periods, and then changed rapidly as desired, even in mobile or portable apparatus.

The vessels of the invention may further comprise means for observing reactions being effected therein, such as fluorescence or luminescence monitoring devices. These may be connected to the reaction vessels by one or more light conducting elements such as optical fibres.

Thus in a further preferred embodiment, the reaction vessel of the invention further comprises one or more signal monitoring means, such as optical fibres or tubes, attached thereto.

The arrangement of the reaction vessel of the invention is such that different signals may be measured from different parts of the reaction at the same time. This may be particularly useful when for example, the monitoring of fluorescent signals at different wavelengths is required. Such monitoring may be of assistance in quantitative amplification reactions such as the TAQMAN™ reactions, or other similar assays for example as described in copending British Patent Application Nos. 9725197.9 and 9725237.3. For

instance, for reactions which include both a quencher and a reporter molecule, there may be advantages to monitoring to the signals generated by each molecule simultaneously in that this may yield more information about the progess of the reaction and allow quantitation of the amplified sequence to be effected.

A further aspect of the invention provides apparatus for carrying out reactions at controlled temperatures, which apparatus comprises a reaction vessel as defined above and a means for controlling the temperature thereof.

Reaction vessels and apparatus of the invention can be used in a variety of situations where chemical or biochemical reactions, or the culture of microrganisms, are required to be carried out. Thus the invention further provides a method of carrying out a reaction which method comprises heating a collection of reagents in a reaction vessel as defined above.

For PCR reactions, the precise temperature conditions required to achieve denaturation, annealing and extension respectively and the time required to effect these stages varies depending upon the nature and length of the nucleotide being amplified, the nature of the primers used and the enzymes employed etc. The optimum conditions may be determined in each case by the person skilled in the art. Typical denaturation temperatures are of the order of 95°C, typical annealing temperatures are of the order of 55°C and extension temperatures of 72°C are generally of the correct order. When utilising the reaction vessels and apparatus of the invention, these temperatures can rapidly be attained and the rate of transition between temperatures readily controlled.

Embodiments of the present invention will now be described, by way of example, with reference to the accompanying drawings, in which

Figure 1 shows a section through a first embodiment of a reaction vessel of the invention;

Figure 2 shows a section through a different embodiment of a reaction vessel of the invention;

Figure 3 shows a section through yet a further embodiment of a reaction vessel of the invention; and

Figure 4 shows a section through an embodiment of a reaction vessel of the invention which allows reaction monitoring to be effected readily.

The embodiment of Figure 1 comprises a conical container (1) and a cap member (2) which projects into the container (1) so as to define a thin space (3) therebetween. A sealing ring (4) ensures that the cap member (2) effectively closes the container (1). A base portion (5) of the container (1) is flattened and made of an optically clear material so that contents of the space (3) may be observed. A sheath of electrically conducting polymer (6) is provided around the container (1). This is provided with electrical connections which may be connected to a power supply.

In use, reagents are introduced into the container (1) before application of the cap member (2). When the cap member (2) is applied, the reagents become distributed through the space (3). Current is then applied to the electrically conducting polymer sheath in order to heat the reaction vessel at it contents to the desired temperature.

The alternative embodiment of Figure 2 shows as container (7) of generally circular cross section but with a flattened base. In this case, the lid (8) is provided with an upper portion (9), which snap fits onto the container (7). Once again a consistent thin space (10) is formed between the container (6) and the lid (7). If desired the upper portion (9) may comprise a lens which allows enhanced observation of contents of the container. Additionally or alternatively, the projecting portion the lid (8) may comprise an optical waveguide such as a fibre optic, which forms an integral part of the reaction monitoring system.

One of the container (7) or lid (8) may comprise an electrically conducting polymer which is connectable to a power supply (not shown). Alternatively, the container may be provided with a sheath of electrically conducting polymer (not shown).

This embodiment may be employed in a similar manner to the embodiment of Figure 1 above.

The modification shown in Figure 3 includes a differently shaped container (11) with a correspondingly differently shaped lid (12) which snap fits onto the container (11). In this case however, the lid (12) includes a channel (13) which can accommodate a temperature monitoring device (14) such as a thermocouple or resistive temperature device (RTD), in order to allow the temperature of the reaction being effected in the container (11) to be monitored.

Again, the container (11) and/or the lid (12) may comprise an electrically conducting polymer, or a sheath of electrically conducting polymer may be provided around the container (11).

The embodiment of Figure 4 illustrates a modification whereby the reaction effected in the vessel may be monitored readily. In this instance the container (15) is generally cylindrical in shape but has an annular projection (16) extending from the base surface thereof. A lid (17) is adapted to sit directly on the base of the container (15) such that the space defined therebetween is generally cylindrical with an annular extension (18). The container (15) may then be surrounded by a sheath of electrically conducting polymer for heating, and the vessel may optionally be placed in a cooling apparatus (not shown).

If the container is illuminated in the direction of the broad arrows, for example using a fluorescent excitation source, any sample in the container will be illuminated. Signal generated by the source may be monitored by an appropriate

fluorescence monitoring device which is arranged in line with the projection (15) in the direction of the line arrows.

Various signals can be monitored simultaneously from different points around the annular projection (15). Alternatively, one or more capillary-like projection may be provided in place of the annular projection (15) so that different signals can be monitored from each. For instance, fluorescence at different wavelengths can be monitored. This may be the wavelengths of for example a reporter and a quencher molecule when these are used together in a reaction such as a TAQMAN™ reaction.

CLAIMS

1. A reaction vessel according to the present invention comprises a container, a cap member, and an electrically conducting polymer which is arranged so as to heat reagents in the reaction vessel when current is supplied to said polymer, the cap member being formed so as to project into the container to reduce the capacity thereof and to create a space therebetween of substantially consistent proportions.

2. A reaction vessel according to claim 1 wherein the cap member is adapted to seal the container.

3. A reaction vessel according to claim 1 or claim 2 wherein the cap member is adapted to snap fit onto the container.

4. A reaction vessel according to any one of the preceding claims wherein the container comprises a glass or plastics material.

5. A reaction vessel according to any one of the preceding claims wherein the container comprises a transparent material.

6. A reaction vessel according to any one of the preceding claims wherein the space created between the cap member and the container when the cap member is in place is of substantially similar proportions to a capillary tube.

7. A reaction vessel according to any one of the claims 1 to 5 wherein the space defined between the container and the lid member is from 0.4 to 1.2mm at any point.

8. A reaction vessel according to any one of the preceding claims wherein the vessel is right cylindrical, conical or paraboloid in form or has an outwardly domed base.

9. A reaction vessel according to claim 8 which is right cylindrical in form.

10. A reaction vessel according to claim 9 wherein the cap member is arranged to impinge upon the base so that the space created when the cap member is in place has the form of an open cylinder.

11. A reaction vessel according to any one of the preceding claims wherein the container comprises a reaction tube or curvette.

12. A reaction vessel according to any one of the preceding claims wherein the cap member is adapted to receive reaction monitoring device.

13. A reaction vessel according to claim 12 wherein the cap member is provided with an opening which can accommodate a temperature measuring device.

14. A reaction vessel according to claim 13 wherein the temperature measuring device is a thermocouple.

15. A reaction vessel according to any one of the preceding claims which further comprises one or more means adapted to observe the development of a signals.

16. A reaction vessel according to claim 15 wherein the said means comprises one or more capillary like extensions from the region of the container in which the space between the cap member and the container is defined.

17. A reaction vessel according to claim 15 wherein the said means comprises an optical fibre.

18. A cap member adapted for use in a reaction vessel according to any one of the preceding claims.

19. Apparatus for carrying out reactions at controlled temperatures, which apparatus comprises a reaction vessel as claimed in any one of claims 1 to 18 and a means for

controlling the supply of current to the electrically conducting polymer so as to temperature thereof.

20. Apparatus according to claim 19 which comprises a plurality of reaction vessels, and further comprises means for controlling the temperature of each reaction vessel independently.

21. Apparatus according to claim 19 or claim 20 which further comprises means for observing a signal generated in the space between the container and the cap member of the reaction vessel.

22. Apparatus according to claim 21 wherein said signal observing means comprises a fluorescence monitoring device.

23. Apparatus according to claim 22 wherein the device is arranged to read fluorescent signal at more than one wavelength.

24. A method of carrying out a reaction which method comprises heating a collection of reagents in a reaction vessel as claimed in any one of claims 1 to 18 or in apparatus as claimed in any one of claims 19 to 23.

25. A method according to claim 24 which is a polymerase chain reaction.

26. A method according to claim 25 which is effected in the presence of more or more labelling agents.

27. A reaction vessel as claimed in claim 1 and substantially as hereinbefore described with reference to the accompanying drawings.

28. Apparatus for reactions according to claim 19 and substantially as hereinbefore described.

The Patent Office

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INVESTOR IN PEOPLE

Application No:	GB 9900697.5	**Examiner:**	R E Hardy
Claims searched:	ALL	**Date of search:**	13 May 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): B1X (X20 X8)

Int Cl (Ed.6): B01J (19/18 19/24); B01L (3/00 3/14 7/00)

Other: Online : EPODOC,WPI,JAPIO

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	GB2181843 A	KUREHA : Whole document	1
A	EP0757246 A2	UNIVERSITAT ROVIRO : Whole document	1
A	EP0245994 A2	ICI AUSTRALIA : Whole document	1
A	US4878597 A	HAAST : Whole document	1
A	WO98/24548 A1	SECRETARY OF STATE : Whole document	1
A	WO89/00755 A1	RAYCHEM : Whole document	1
A	DE003132926 A	MEDIZIN : Whole document	1

X Document indicating lack of novelty or inventive step
Y Document indicating lack of inventive step if combined with one or more other documents of same category.
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A Document indicating technological background and/or state of the art.
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